CCXIX. THE OXIDATION OF PHENYL DERIVATIVES OF FATTY ACIDS WITH HYDROGEN PEROXIDE IN THE PRESENCE OF COPPER.

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The method and extent of the oxidation of the phenyl derivatives of the fatty acids *in vitro* is of especial interest since it is largely on the results of feeding experiments carried out with these acids that Knoop's theory of the β-oxidation of the fatty acids in the body has been built up. In the present investigation we examined the influence of a cupric salt on the oxidation of phenyl derivatives of the fatty acids by means of hydrogen peroxide: an excess of the hydrogen peroxide was run into the mixture of sodium salt of the phenyl-substituted fatty acid with a small proportion of cupric salt, as in the method already described for the oxidation of fatty acids [Smedley-MacLean and Pearce, 1934]. At 90° the phenyl derivatives were much more rapidly attacked than the fatty acids themselves and it was found that even at 60° in less than an hour the acid in some cases was almost entirely converted into carbon dioxide: no attempt was made to keep either the concentration of the peroxide or the p_H constant.

The results of the oxidation of the fatty acids under parallel conditions are also given in Table I. In the absence of copper benzoic acid was almost unattacked at 60°, formic being rather better oxidised: propionic and butyric acids were almost unattacked; in the aromatic acids the extent of the oxidation increased with the length of the side-chain.

The addition of the cupric salt increased the amount of oxidation in all cases but most markedly in the aromatic series: formic and benzoic acids were readily attacked but the most vigorous action occurred with phenylacetic acid, for in less than half an hour 80 % of this acid was oxidised to CO_2. A few minutes after the reacting substances were mixed together there was a rush of carbonic acid through the absorption apparatus, the effect being most striking when the peroxide and sodium phenylacetate were heated at 60° and the cupric salt solution then run in. The special susceptibility of phenylacetic acid to oxidation is possibly due to the fact that β-oxidation would take place at the nuclear carbon atom to which the side-chain is attached.

Both in the presence and absence of the catalyst, the unsaturated cinnamic acid was less readily oxidised than phenylpropionic acid.

In order to throw some light on the intermediate stages of the reaction, a series of experiments was carried out in which only limited amounts of hydrogen peroxide were added: at the end of the hour's heating, the residual solutions gave positive tests for the presence of phenolic compounds: qualitative tests with solutions of ferric chloride, silver nitrate, potassium cyanide, alkali and gelatin demonstrated the presence of polyhydroxy-compounds amongst which gallic acid was positively identified. The carbon content of the organic matter extracted by ether from the residual solution was 51·6 %, theory for gallic acid requiring 49·4 %.

Table I.

Oxidation of aliphatic acids and phenyl-substituted fatty acids with hydrogen peroxide at 60° for one hour (A) without a catalyst, (B) with a copper catalyst.

ALIPHATIC ACIDS.

Percentage of carbon as		Formic acid	Acetic acid	Propionic acid	Butyric acid
A	Unchanged acid	88·0	—	98·0	99·0
	Carbon dioxide	10·2	—	0·0	1·0
B	Unchanged acid	11·3	65·0	50·0	82·0
	Carbon dioxide	96·1	31·3	25·2	7·9
	Aldehyde and ketone	0·0	0·0	1·9*	5·9†
	Formic acid	—	2·4	6·0	1·9
	Succinic acid	—	0·0	—	1·2
	Oxalic acid	—	—	1·4	—

* Acetaldehyde. † Acetone and aldehyde.

PHENYL-SUBSTITUTED ACIDS.

Percentage of carbon as		Benzoic acid	Phenyl-acetic acid	Phenyl-propionic acid	Cinnamic acid	Phenyl-butyric acid
A	Unchanged acid	99·8	80·0	76·6	72·0	61·2
	Carbon dioxide	0·2	4·0	11·0	1·5	8·5
	Aldehyde and ketone	Trace	Trace*	Trace†	Trace	0·7‡
	Formic acid	0·0	0·9	0·8	1·9	1·1
	Acetic acid	—	10·5	—	—	7·4
	Succinic and oxalic acids	—	—	—	—	Present
B	Unchanged acid	0·2	1·8	3·0	5·1	1·5
	Carbon dioxide	62·6	77·4	53·2	27·1	21·4
	Aldehyde and ketone	0·5*	0·3	0·6†	1·0†	1·5
	Formic acid	4·7	3·3	1·7	7·9	10·3
	Acetic acid	3·3	8·1	—	6·1	6·5
	Succinic acid	—	—	6·0	—	42·0
	Oxalic acid	Present	3·8	4·7	—	22·0

* Formaldehyde. † Benzaldehyde. ‡ Acetone.

Dakin and Herter [1907] showed that by the oxidation of phenylalanine with hydrogen peroxide hydroxy-groups were introduced into the aromatic nucleus and that the action of hydrogen peroxide on benzoic acid resulted in the formation of salicylic acid with smaller quantities of *m*- and *p*-hydroxybenzoic acids. There was also evidence of the presence of dihydroxy-compounds. Indications were obtained that the reaction proceeded to some extent at laboratory temperature, but in the actual experiments described the reaction mixture was heated for some hours at 100°. β-Phenylpropionic acid yielded β-phenylhydroxypropionic acid, acetophenone being also identified.

Traube [1910] showed that polyhydroxy-aliphatic compounds were oxidised by hydrogen peroxide in the presence of copper, a complex cupric ion being formed. Since hydroxylation of the nucleus occurred in the absence of copper the cupric salt probably reacts with the phenolic groups, as Weinland and Walter [1923] have shown happens in the case of catechol, and exercises a potent effect on the disruption of the benzene nucleus. That, in the presence of copper, it is the nucleus which is the chief point of attack seems also indicated by the fact that the oxidation is less potent with increasing length of side-chain, whereas in the absence of copper the reverse effect occurs.

The formation of succinic acid from phenylpropionic and phenylbutyric acids would indicate that oxidation had taken place in the γ-position. No evidence of the presence of any ketone was obtained. As in the case of the fatty acids [Smedley-MacLean and Pearce, 1934] the introduction of the cupric salt led to the formation of hydroxy- and not of keto-compounds.

Salkowski [1879] showed that phenylacetic acid was not oxidised in the organism but was excreted as phenylaceturic acid, whereas phenylpropionic acid was oxidised to benzoic acid and then eliminated as hippuric acid. Knoop [1904] extended this work and showed that when the phenyl derivatives of the fatty acids were fed to dogs, if the acid contained an even number of carbon atoms in the side-chain, phenylacetic acid appeared in the urine: if the side-chain contained an odd number of carbon atoms benzoic acid was eliminated, both the phenylacetic and benzoic acids being coupled with glycine. Quick's results [1926] make it probable that these acids are almost entirely eliminated either in combination with glycine or glycuronic acids. Dakin [1908] tested the hypothesis that the resistance of these acids to oxidation in the organism might be due to the protective action of the coupling with glycine: he carried out feeding experiments in which phenylpropionylglycine was fed: no quantitative data are given but the normal oxidation products of the uncoupled acid were detected in the urine, amongst them cinnamoylglycine. Since in the body however the coupling either does not take place or does not become protective until the stage of benzoic or phenylacetic acid has been reached, we carried out experiments to ascertain whether coupling with glycine affected the oxidation of these acids with hydrogen peroxide in the presence of copper and found that a marked degree of protection was accorded. The results are recorded in Table II.

Table II. *Percentage of carbon evolved as carbon dioxide during oxidations by means of hydrogen peroxide for one hour at 60° with a copper catalyst.*

	Experimental value	If coupled compound behaves as a mixture		Experimental value
Acetic acid Glycine	31·3 45·2	38·2	Aceturic acid	15·3
Benzoic acid Glycine	63·6 45·2	59·0	Hippuric acid	0·2
Phenylacetic acid Glycine	77·4 45·2	70·7	Phenylaceturic acid	39·0

We know nothing as to how this coupling is effected in the body, but the fact that coupling with glycine almost entirely prevents the oxidation of benzoic acid by hydrogen peroxide in the presence of a copper catalyst would support the view that the coupling acts as a protective mechanism.

Summary.

1. H_2O_2 in the presence of a cupric catalyst rapidly oxidised the phenyl derivatives of the fatty acids, phenolic compounds being formed as intermediate stages.

2. In the absence of the catalyst, with increasing length of side-chain, more of the acid was oxidised; in its presence phenylacetic acid was most readily attacked, 80 % of its carbon being converted into CO_2 in less than half an hour at 60°.

3. Coupling with glycine rendered acetic, benzoic and phenylacetic acids less susceptible to oxidation, hippuric acid being practically unattacked under the conditions of oxidation used.

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